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2,3-Pentanedione

MAK Value Documentation – Translation of the German version from 2017

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Keywords

2,3-pentanedione, lung toxicity, nose, irritation, maximum workplace concentration, MAK value, peak limitation, skin sensitization, skin absorption

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has evaluated 2,3-pentanedione [600-14-6] to derive a maximum concentration at the workplace (MAK value), considering all toxicity endpoints. The critical effects of 2,3-pentanedione were inflammation, necrosis, ulceration and fibrosis in the lung and inflammation, exudates and metaplasia in the nasal cavity in rats and mice after inhalation for 14 days. In this study the NOAEC in rats was 49 ml/m^3 and the LOAEC in mice was 49 ml/m^3 for effects in the lung. The occurrence of fibrosis after only two weeks of inhalation is assessed as a severe effect. Due to the structural similarity of 2,3-pentanedione to diacetyl (2,3-butanedione), which is responsible for bronchiolitis obliterans in popcorn workers, and the likeliness of lung effects in rodents, a MAK value of 0.02 ml/m^3 is set in analogy to diacetyl. As the critical effect is systemic, 2,3-pentanedione is assigned to Peak Limitation Category II. The excursion factor of 1 is set in analogy to diacetyl. Skin contact may contribute significantly to systemic toxicity and 2,3-pentanedione is designated with an "H". In analogy to diacetyl, skin sensitization (Sh) is expected but not airway sensitization. Because there are no studies on developmental toxicity, the substance is assigned to Pregnancy Risk Group D. 2,3-Pentanedione is neither genotoxic nor carcinogenic.

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MAK value (2016)	**0.02 ml/m³ (ppm) ≙ 0.083 mg/m³**
Peak limitation (2016)	**Category II, excursion factor 1**
Absorption through the skin (2016)	**H**
Sensitization (2016)	**Sh**
Carcinogenicity	**–**
Prenatal toxicity (2016)	**Pregnancy Risk Group D**
Germ cell mutagenicity	**–**
BAT value	**–**
Synonyms	acetyl propionyl
Chemical name	2,3-pentanedione
CAS number	600-14-6
Structural formula	$CH_3–CH_2–CO–CO–CH_3$
Molecular formula	$C_5H_8O_2$
Molar mass	100.12 g/mol
Melting point	−52 °C (NIOSH 2011)
Boiling point	108–112 °C (NIOSH 2011)
Density at 19 °C	0.9565 g/cm³ (NIOSH 2011)
Vapour pressure at 20 °C	28.5 hPa (NIOSH 2011)
log K_{OW}	−0.85 (NIOSH 2011)
Solubility	60 000 mg/l water at 15 °C (NIOSH 2011)
1 ml/m³ (ppm) ≙ 4.158 mg/m³	**1 mg/m³ ≙ 0.240 ml/m³ (ppm)**

2,3-Pentanedione is used as an artificial flavouring mainly to give foods a buttery taste. The substance is used in microwave popcorn, beverages, ice cream, biscuits, cakes and pastries, and custards. It can be found as a natural flavour in beer, wine and yoghurt. In addition, it is released when coffee is roasted (Boylstein 2012; Morgan et al. 2012 b).

The presence of 2,3-pentanedione was demonstrated in the air at workplaces in dry bakery mix production, where various buttermilk flavourings are used. 2,3-Pentanedione concentrations of 0.078 ml/m³ (workplace air) and 0.091 ml/m³ (personal sampling) were determined using the OSHA 1013 method, which is considered to be unbiased. All other measured concentrations were below the limit of quantification of 0.069 ml/m³ (Day et al. 2011).

The concentrations of 2,3-pentanedione in 105 ambient air samples and 74 samples collected by personal sampling from nine food production plants and one flavour manufacturer were below the limit of detection (LOD) of 0.2 to 1 µg 2,3-pentanedione/sample in most cases. Only in three samples from a production facility for cereals were the

concentrations 0.015 to 0.172 ml 2,3-pentanedione/m³. Here also, the OSHA 1013 method was used (Curwin et al. 2015).

In the USA, 2,3-pentanedione concentrations of 22.8 to 72.5 ml/m³ were found in the mainstream smoke from filter cigarettes. This corresponds to 29.2–165 µg/cigarette. During the smoking process, various tobacco components and the cigarette paper pyrolyse to form 2,3-pentanedione and diacetyl. In Germany, diacetyl is allowed as an additive up to 0.00001% by weight in cigarettes, in Great Britain, the permissible 2,3-pentanedione content as an additive is up to 0.001% by weight (Pierce et al. 2014 a).

While roasting coffee, 2,3-pentanedione concentrations of up to 0.018 ml/m³ occurred, and during grinding, concentrations of between 0.0089 and 0.21 ml/m³. The detection limit was 0.0011 to 0.0032 ml 2,3-pentanedione/m³. The presence of the structurally similar diacetyl was also demonstrated (Gaffney et al. 2015).

In another coffee-roasting facility, two cases of bronchiolitis obliterans were described in employees after less than 18 months at the workplace. The exposure was not determined (Huff et al. 2013).

The odour threshold for 2,3-pentanedione is 10 to 20 µg/m³ (0.0024–0.0048 ml/m³) (NIOSH 2011).

1 Toxic Effects and Mode of Action

On rabbit skin, pure 2,3-pentanedione causes moderate irritation after occlusive application.

Changes in the composition of the bronchoalveolar lavage fluid (BALF) in the lungs were found in mice and rats after only 5 exposures to 2,3-pentanedione at a concentration of 200 ml/m³ for six hours. After 12 six-hour exposures to 49 ml/m³, inflammation and exudate in the nasal cavity were observed in mice and rats. In mice, also atrophy of the olfactory epithelium and inflammation in the larynx were found. In addition, inflammation of the lungs occurred in mice even at this concentration level, whereas in rats, regeneration of the lungs was found as the initial effect only at the next-higher concentration of 97 ml 2,3-pentanedione/m³. The effects are concentration-dependent. After only 12 six-hour exposures, histopathology of the rat lung revealed bronchial fibrosis at 2,3-pentanedione concentrations of 150 ml/m³ and epithelial ulceration and regeneration at 200 ml/m³.

Investigation of the gene expression of fibrotic tissue in rats exposed to 2,3-pentanedione revealed the participation of pro-fibrotic mediators and genes involved in reconstruction of the extracellular matrix.

In bacterial mutagenicity tests, 2,3-pentanedione was not found to be mutagenic. In vivo, no increase in micronuclei in the peripheral blood of rats and mice after exposure to 100 ml 2,3-pentanedione/m³ for 13 weeks was recorded.

2,3-Pentanedione has sensitizing effects on the skin of mice. There are no studies available for the carcinogenicity or reproductive toxicity of the substance.

2 Mechanism of Action

There are no mechanistic studies with 2,3-pentanedione available.

Like diacetyl, 2,3-pentanedione belongs to the group of α-dicarbonyls, which are chemically very reactive (Hubbs et al. 2012).

The reactivity of diacetyl is based on the following properties: The electron affinity of diacetyl is similar to that of quinones and dinitrophenol, which are likewise electron acceptors. Diacetyl is capable of binding to biomolecules and forming conjugated imines with primary amines, and is able to induce protein/protein crosslinks (Kovacic and Cooksy 2010). Furthermore, diacetyl reacts with cysteine to form cyclic products such as thiazoles, thiazolines, oxazoles and pyrazines (Marchand et al. 2011). Diacetyl reacts with the guanidine group of arginine. This modification of arginine represents the formation of a hapten and can result in a specific antibody response (Mathews et al. 2010). Diacetyl forms adducts with 2-deoxyguanosine (More et al. 2012).

As a result of the structural similarity of 2,3-pentanedione and diacetyl, both of which carry the same functional group, 2,3-pentanedione can be assumed to have the same toxic effects on the respiratory tract and a similar mechanism. It is difficult to compare the relative toxic potency of both substances because of the different durations of exposure in the documented experiments. An attempt to calculate their relative toxic potency revealed that diacetyl has a slightly higher potency in female mice. However, the authors assume that both substances have a roughly similar effect (Day et al. 2011; NIOSH 2013 Table 6.10). In the lungs, diacetyl induces inflammation that is capable of producing an irreversible obstruction of the respiratory tract. In persons employed in the food industry, where diacetyl is used as a butter flavouring, the otherwise very rare disease bronchiolitis obliterans has been diagnosed numerous times (Hartwig and MAK Commission 2016). The damage occurring in the pulmonary epithelium of rats after exposure to 2,3-pentanedione corresponded to the histopathological effects observed with bronchiolitis obliterans (King 1989; Morgan et al. 2008, 2012 b; NIOSH 2013).

In the opinion of the Commission, no further systemic effects are to be expected from diacetyl as, because of its high reactivity, it can be assumed that it immediately reacts with the surface of the lungs (Hartwig and MAK Commission 2016). This is also assumed for 2,3-pentanedione.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

As rodents breath through the nose, the amount of diacetyl reaching the lungs is markedly lower than that in humans, who, during physical exercise, also breath through the mouth, which results in an increased uptake (Hartwig and MAK Commission 2016; Morris and Hubbs 2009). By analogy to diacetyl, this also applies for 2,3-pentanedione (Hubbs et al. 2012).

Ingested α-diketones are rapidly absorbed and metabolized in the gastrointestinal tract (WHO 1999).

Neither in vivo nor in vitro data are available for the absorption of 2,3-pentanedione through the skin. Using the models of Fiserova-Bergerova et al. (1990), Guy and Potts (1993) and Wilschut et al. (1995), fluxes of 48.1, 6.63, and 14.9 µg/cm^2 and hour, respectively, are calculated for a saturated aqueous solution. Assuming the exposure of a 2000 cm^2 surface area of skin for one hour, this would correspond to absorbed amounts of 96.2, 13.3 and 29.8 mg, respectively.

3.2 Metabolism

There are no data available for the metabolism of 2,3-pentanedione.

α-Diketones and the corresponding ketoenol are in equilibrium (WHO 1999).

In analogy to the acetaldehyde metabolites acetoin (CH_3–CO–CHOH–CH_3) and diacetyl, it can be assumed that after the ingestion of 2,3-pentanedione the metabolite CO_2 is produced by an oxidation reaction, some of which is exhaled. To date, this has been demonstrated only for acetoin. In Sprague Dawley rats given intracardial injections of acetoin-2,3-^{14}C (CH_3-*CO-*CHOH-CH_3), 47% of the radioactivity was exhaled in the form of $^{14}CO_2$ within 20 hours. Intraperitoneally injected acetoin-2,3-^{14}C and acetate-1-^{14}C were converted into 15 and 45% $^{14}CO_2$, respectively, in the rats (no other details, presumably exhaled). In the urine, 13% of the administered radioactivity (the radioactive compound was not identified) was recovered after 12 hours. The incubation of acetoin-2,3-^{14}C or acetoin-1,4-^{14}C with a micronized liver preparation resulted likewise in the formation of $^{14}CO_2$. The production of $^{14}CO_2$ from acetoin-1,4-^{14}C was slower here by a factor of 5 (Gabriel et al. 1972).

4 Effects in Humans

There are no data available for the following end points: single exposures, local effects on skin and mucous membranes, reproductive and developmental toxicity, genotoxicity and carcinogenicity.

The OSHA 1016 analytical method used to determine the concentration of 2,3-pentanedione in air has a detection limit of 0.0093 ml/m³ with a standard deviation of 10.1% (OSHA 2010). In some publications, the concentrations of 2,3-pentanedione were determined using the analytical methods OSHA 1012 or 1013 for diacetyl. These methods are likewise considered to be unbiased (OSHA 2008 a, b).

4.1 Repeated exposure

There are no studies available for repeated exposure to 2,3-pentanedione at the workplace.

In workers employed in food production, an increased incidence of bronchiolitis obliterans occurred, the cause of which was identified as the structural analogue diacetyl (Hartwig and MAK Commission 2016).

In human medicine, the term bronchiolitis obliterans is used for two types of lesions. In the first case, intraluminal polyps are observed. This type was formerly designated as BOOP (bronchiolitis obliterans-organizing pneumonia). It is characterized by polypoid protrusions of the granular tissue inside the lumen of the bronchioles, the alveolar ducts and the alveoli. A second type is frequently designated as constrictive bronchiolitis and is characterized by intramural fibrosis with a narrowing of the lumen (Morgan et al. 2012 b).

The concentrations of various aldehydes and ketones were determined in the mainstream smoke of six different cigarette brands. The mean 2,3-pentanedione concentrations were in the range of 32.2 to 50.1 ml/m³. This means that a pack-year corresponds to a mean cumulative exposure to 2,3-pentanedione of 0.14 to 0.26 ml/m³ × year ("ppm-year"). Smoking is not considered to be a risk factor for bronchiolitis obliterans. In the authors' opinion, the exposure to 2,3-pentanedione (and diacetyl) from cigarette-smoking far exceeds that determined at the workplace. The lacking quantification of 2,3-pentanedione (and diacetyl) inhaled from cigarette smoke therefore represents a confounder which, in the authors' opinion, has not been taken into consideration adequately in the studies of exposure at the workplace. In the light of these results the authors call into question whether 2,3-pentanedione and diacetyl can be considered as inducers of bronchiolitis obliterans (Pierce et al. 2014 a, b). Other authors highlight the cluster of cases of a very rare disease and thus consider diacetyl or 2,3-pentanedione to be a causal factor. They emphasize that smoking has been taken into account. A further argument is that exposure is higher after inhalation for 8 hours during a working day than after short-term smoking (Akpinar-Elci and Elci 2014; Farsalinos et al. 2015 b).

2,3-Pentanedione has also been found in sweet-flavoured electronic cigarette liquids. The median exposure was 91 µg/day (interquartile range 20–432 µg/day) (Farsalinos et al. 2015 a; Hubbs et al. 2015).

Summary: In workers in food production, the disease bronchiolitis obliterans occurred with a greater frequency than normal, for which diacetyl, which is structurally similar to 2,3-pentanedione, was identified as the cause (Hartwig and MAK Commission 2016). During one pack-year, cigarette smoking leads to a mean cumulative exposure to 2,3-pentanedione of 0.14 to 0.26 ml/m³ × year (Pierce et al. 2014 a). The median uptake from smoking an electronic cigarette was 91 µg 2,3-pentanedione/day (Farsalinos et al. 2015 a). The NIOSH (2011) established a limit value of 0.0091 ml/m³ (0.039 mg/m³) for 2,3-pentanedione, which corresponds to an uptake of 390 µg/day with an assumed respiratory volume of 10 m³. The contribution of smoking to the occurrence of the severe obstructive pulmonary disease bronchiolitis obliterans is unclear. To date, smoking has not been shown to be a risk factor for bronchiolitis obliterans.

4.2 Allergenic effects

Unlike in the case of diacetyl (Hartwig and MAK Commission 2016), no clinical findings are available for contact sensitizing effects of 2,3-pentanedione. Also, no data are available for respiratory sensitization. Maximization tests by Epstein in 1976 and 1977 with 4% petrolatum preparations of 2,3-pentanedione and the homologous 2,3-hexanedione did not produce sensitization in any of the 25 volunteers in either case (Author not named 1979 a, b). On the other hand, 2 of the 25 volunteers reacted to a 5% preparation of 2,3-heptanedione in a maximization test carried out by Kligman in 1978 (FEMA 2011).

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

In an inhalation study with 6-hour whole-body exposure to 2,3-pentanedione concentrations of 0, 111.7, 241.2, 318.4 or 354.2 ml/m³ (purity 97%), 4 of 6 male Hla:(SD)CVF rats in the group given 318.4 ml/m³ displayed clinical abnormalities in the form of breathing through the mouth with respiratory noises (1/6), rales during inspiration (2/6) and an increased respiratory frequency (1/6) 18 hours after the end of exposure. In the high concentration group, the following clinical changes occurred in 5 of 6 animals 18 hours after the end of exposure: breathing through the mouth (3/6), breathing noises (3/6), the absence of lung sounds (1/6) and increased swallowing (1/6). No unusual findings were observed in the two low concentration groups. Histopathology revealed significantly increased necrosis and apoptosis of the respiratory and olfactory epithelium of the nose in all concentration groups. In the low concentration group, these findings were confined to the first section level. At concentrations of 241.2 ml/m³ and above, an increase in neutrophils in the nasal secretion was observed. In the olfactory neuroepithelium of the exposed rats, apoptosis and multifocal invaginations occurred in the second section level, and at the high concentration also in the third section level. In the lungs, the observed effects were restricted to the stem bronchi. Here, the incidence of apoptosis was minimal but statistically significant at the two high concentrations, and increased significantly in the 12 to 18-hour recovery periods. At 2,3-pentanedione concentrations of 241.2 ml/m³ and above, the spacing between the olfactory neurons of olfactory neuroepithelium was irregular, the olfactory neurons were small, there was a loss of the connective tissue cells and the dendrites of the olfactory neurons were thickened and shortened in the exposed rats. In addition, foci of disorganized neurons occurred besides the foci of invaginations of the olfactory neuroepithelium. In a parallel group of rats whole-body exposed to diacetyl at a concentration of 240 ml/m³, no clinical abnormalities were found. The histopathological changes in the respiratory epithelium after exposure to 2,3-pentanedione and diacetyl concentrations of 241.2 ml/m³ and 240 ml/m³, respectively, were similar. In another experiment, 6 male Hla:(SD)CVF rats were exposed to 2,3-pentanedione at a concentration of 270 ml/m³ for 6 hours and 41 minutes. Using real-time PCR, increased expression of IL-6 and NO-synthase-2 (NOS-2) and a reduction in the expression of vascular endothelial growth factor A (Vegf-A) were found in different regions of the brain (bulbus olfactorius, striatum, hippocampus, cerebellum). An increased, 2,3-pentanedione-mediated, expression of blood-brain barrier marker Cldn1 was observed only in the bulbus olfactorius. Based on these findings, the authors concluded that 2,3-pentanedione has neurotoxic effects similar to those obtained with 2,4-pentanedione (MAK value 20 ml/m³) (Greim 2007, available in German only; Hubbs et al. 2010, 2012).

Inhalation exposure to 2,3-pentanedione concentrations of 0, 118, 242, 315 or 356 ml/m³ for 6 hours with an 18-hour recovery period did not affect lung resistance and dynamic lung compliance in groups of 4 to 10 male Sprague Dawley rats. The administration of 20 µl of a methacholine solution (10 mg/ml, highest tested dose) in the form of an aerosol after the recovery period led to decreased lung resistance and consequently to bronchodilation in all treated groups. At 242 ml/m³ there was a marked increase in dynamic lung compliance, but not at higher concentrations (Zaccone et al. 2013).

5.1.2 Oral administration

The oral LD_{50} was 3000 mg 2,3-pentanedione/kg body weight in rats (no other details; EFSA 2014).

5.1.3 Dermal application

The dermal LD_{50} value was > 2500 mg 2,3-pentanedione/kg body weight in rabbits (no other details; Author not named 1979 b).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

All the results from the studies described below are given in detail in Table 1.

Tab. 1 Findings in the respiratory tract of rats and mice after inhalation of 2,3-pentanedione

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, 6 ♂	**1, 3, 5, 10 exposures,** 0, 50,100, 200 ml/m³, 6 hours/day, 5 days/week, whole-body exposure	**100 ml/m³: NOAEC**; **200 ml/m³**: **5 exposures**: BALF: PMN ↑*, cytokines ↑ (CRP*, fibrinogen*, FGF-9, MCP-1, MCP-3*, OSM); **10 exposures**: BALF: PMN ↑*, cytokines ↑ (CRP*, fibrinogen*. FGF-9*, MCP-1*, MCP-3*, OSM)	Morgan et al. 2012 b
rat, Wistar, 6 ♂, 6 ♀	**12 exposures**, 0, 49, 97, 202 ml/m³, 6 hours/day, 5 days/week, whole-body exposure	**49 ml/m³: LOAEC**, nasal cavity: mucosal inflammation (♂: 4/6 mild, ♀: 5/6 minimal), exudate (♂: 4/6 mild, ♀: 3/6 minimal), respiratory epithelium: squamous metaplasia (♂: 2/6 mild), regeneration (♂: 6/6 minimal, ♀: 5/6 minimal), olfactory epithelium: atrophy (♂: 1/6 minimal), degeneration (♀: 1/6 minimal); **97 ml/m³**: nasal cavity: mucosal inflammation (♂: 6/6 mild to moderate, ♀: 6/6 minimal), exudate (♂: 6/6 mild, ♀: 5/6 minimal), respiratory epithelium: necrosis (♂: 1/6 minimal), ulceration (♂: 1/6 minimal), squamous metaplasia (♂: 6/6 mild, ♀: 5/6 slight), regeneration (♂: 6/6 moderate, ♀: 4/6 mild), olfactory epithelium: atrophy (♂: 6/6 mild, ♀: 2/6 minimal to mild), degeneration (♂: 3/6 minimal, ♀: 5/6 minimal), lungs: minimal regeneration of the bronchial epithelium (12/12); **202 ml/m³**: **1 exposure**: 5 animals died (4 ♂, 1 ♀), **5 exposures**: body weights ↓* (♂: 31%, ♀: 23%), **12 exposures**: body weights ↓* (♂: 30%, ♀: 24%), nasal cavity: mucosal inflammation (♂: 2/2 mild to moderate, ♀: 3/4 mild to moderate), exudate (♂: 2/2 mild, ♀: 3/4 mild), respiratory epithelium: necrosis (♂: 1/2, ♀: 3/4), ulceration (♂: 1/2, ♀: 2/4), squamous metaplasia (♂: 2/2, ♀: 2/4), olfactory epithelium: atrophy (♂: 2/2 moderate, ♀: 3/4 mild), degeneration (♂: 2/2 minimal to mild, ♀: 3/4 mild), larynx: necrosis, ulceration, focal squamous metaplasia, lungs: inflammation (♂: 2/2 mild, ♀: 4/4 mild), necrosis (♂: 2/2 moderate, ♀: 4/4 mild), ulceration (♂: 2/2 mild, ♀: 4/4 mild), regeneration (♂: 2/2 mild, ♀: 4/4 moderate), bronchial fibrosis (♂: 2/2, 11 fibrotic lesions per animal, ♀: 3/4, 8–26 fibrotic lesions per animal), intraluminal fibrosis (♂: 2/2 moderate, ♀: 4/4 moderate), intramural fibrosis (♂: 1/2 minimal, ♀: 4/4 minimal), ♀: squamous metaplasia 3/4, ♂: relative lung weights ↑*, **See text for description of pulmonary lesions**	

Tab. 1 *(continued)*

Species, strain, number per group	Exposure	Findings	References
rat, Wistar Han, 5–6 ♂	**12 exposures**, 0, 100, 150, 200 ml/m³, 6 hours/day, 5 days/week, 1 day recovery period	**150 ml/m³**: airway resistance ↑ n. s., lung compliance ↓ n. s., intraluminal and intramural bronchial fibrosis (3/6); **200 ml/m³**: intraluminal and intramural bronchial fibrosis (5/5)	Morgan et al. 2012 a
rat, Wistar Han, 3–9 ♂	**12 exposures**, 0, 100, 150, 200 ml/m³, 6 hours/day, 5 days/week, 2 weeks recovery period	**150 ml/m³**: intraluminal and intramural bronchial fibrosis (1/9); **200 ml/m³**: intraluminal and intramural bronchial fibrosis (3/3); **only lungs investigated**	
rat, Wistar Han, 5 ♂, controls: 6 ♂	**12 exposures,** 0, 200 ml/m³, 6 hours/day, 5 days/week, whole-body exposure	**controls**: bronchial lesions: inflammation 2/6 (minimum); **200 ml/m³**: bronchial lesions: fibrosis 5/5 (6–10 lesions/animal), epithelial ulceration 5/5 (slight), epithelial regeneration 5/5 (slight), epithelial hyperplasia 5/5 (minimal), inflammation 5/5 (moderate), **See text for analysis of gene expression and description of pulmonary lesions**	Morgan et al. 2015

Tab. 1 *(continued)*

Species, strain, number per group	Exposure	Findings	References
mouse, B6C3F1, 6 ♂	**5 or 10 exposures**, 0, 50, 100, 200 ml/m³, 6 hours/day, 5 days/week, whole-body exposure	**200 ml/m³**: **5 exposures**: BALF: fibrinogen ↑*, GCP-2 ↓*; **10 exposures**: 6–9 exposures: 2 animals died, BALF: PMN ↑*	Morgan et al. 2012 b
mouse, B6C3F1, 6 ♂, 6 ♀	**12 exposures**, 0, 49, 97, 202 ml/m³, 6 hours/day, 5 days/week, whole-body exposure	**49 ml/m³**: **LOAEC**, body weight ↓* (by about 11%, no other details), nasal cavity: inflammation of the mucosa (12/12 minimal to mild), exudate (♂: 3/6 minimal), respiratory epithelium: necrosis (♂: 4/6, ♀: 1/6 minimal), necrosis of the nasal concha (♂: 1/6 minimal), squamous metaplasia (♂: 5/6 mild, ♀: 6/6 minimal), regeneration (♂: 6/6 mild, ♀: 6/6 mild), olfactory epithelium: atrophy (♂: 6/6, mild, ♀: 6/6, mild), larynx: inflammation, epithelial necrosis (some animals affected, no other details), lungs: inflammation (♂: 5/6 minimal, ♀: 4/6 minimum); **97 ml/m³**: body weights ↓* (by about 11%, no other details), nasal cavity: mucosal inflammation (12/12 mild), exudate (♂: 6/6 minimal, ♀: 4/6 minimal), respiratory epithelium: necrosis (♂: 5/6 minimal, ♀: 4/6 minimal to mild), ulceration (♀: 2/6 minimal), necrosis of the nasal concha (♂: 4/6 minimal, ♀: 5/6 minimum), squamous metaplasia (♂: 5/6 mild, ♀: 5/6 minimal to mild), mild to moderate regeneration, olfactory epithelium: atrophy (♂: 6/6 moderate, ♀: 6/6 moderate), larynx: inflammation, necrosis, regeneration, squamous metaplasia (no other details), lungs: inflammation (♂: 6/6 mild, ♀: 6/6 mild), regeneration of the bronchial epithelium (♂: 5/6 minimal, ♀: 2/6 minimal); **202 ml/m³**: **5 exposures**: body weights ↓* (♂: 27%, ♀: 25%), **12 exposures**: body weights ↓* (♂: 31%, ♀: 29%), nasal cavity: inflammation of the mucosa (♂: 6/6 mild, ♀: 5/5, mild to moderate), exudate (♂: 6/6 mild, ♀: 5/5 mild), respiratory epithelium: necrosis (♂: 6/6 mild, ♀: 5/5 moderate), ulceration (♂: 6/6 mild, ♀: 4/5 minimal), necrosis of the nasal concha (♂: 6/6 mild, ♀: 5/5 moderate), squamous metaplasia (♂: 6/6 mild to moderate, ♀: 5/5 mild), mild regeneration, olfactory epithelium: atrophy (♂: 5/6 severe, ♀: 5/5 severe), larynx: inflammation, necrosis, regeneration, squamous metaplasia (no other details), lungs: inflammation (♂: 5/6 moderate, ♀: 5/5 mild), bronchial epithelium: necrosis (♂: 3/6 minimal, ♀: 3/5 mild), ulceration (♂: 3/6 minimal, ♀: 2/5 minimal), regeneration (♂: 5/6 mild, ♀: 3/5 mild), ♂: relative lung weights ↑*, **See text for description of pulmonary lesions**	

*p < 0.05; BALF: bronchoalveolar lavage fluid; CRP: C-reactive protein; FGF-9: fibroblast growth factor-9; GCP-2: granulocyte chemotactic protein-2; LOAEC: lowest observed adverse effect concentration; MCP-1: monocyte chemotactic protein-1; MCP-3: monocyte chemotactic protein-3; NOAEC: no observed adverse effect concentration; n. s.: not significant; OSM: oncostatin M; PMN: polymorphonuclear neutrophils

For 1, 3, 5 or 10 days, groups of 6 male Wistar rats and 6 male B6C3F1 mice were exposed in whole-animal chambers (6 hours/day, 5 days/week) to 2,3-pentanedione concentrations of 0, 50, 100 or 200 ml/m³. In rats, the number of neutrophils (PMN) and the cytokine level in the bronchoalveolar lavage fluid (BALF) were significantly increased at the high concentration after 5 exposures. Under the same exposure conditions, the PMN count was significantly increased only after 10 treatments in male mice of the high concentration group. However, the exposure did not

cause an increase in the total number of cells, in total proteins or in LDH activity in the BALF of either rats or mice (Morgan et al. 2010, 2012 b).

Twelve exposures to 2,3-pentanedione concentrations of 0, 49, 97 or 202 ml/m³ in 6 female and 6 male Wistar rats or B6C3F1 mice (6 hours/day, 5 days/week) produced effects even at the low concentration. In rats, minimal to mild mucosal inflammation and exudate in the nasal cavity occurred in 9 of 12 and 7 of 12 animals, respectively, at 49 ml/m³, and metaplasia of the respiratory epithelium in 2 males. Minimal atrophy or degeneration of the olfactory epithelium were found in one animal in each case. In the lungs, minimal regeneration of the bronchial epithelium was observed at 97 ml/m³. The incidence and severity of the effects were concentration-dependent. Mice were more sensitive: at 49 ml/m³, in addition to the effects in the nasal cavity and the olfactory epithelium, inflammation and necrosis in the larynx were observed and, in 9 of 12 animals, also lung inflammation of minimal severity. These effects likewise increased with the concentration. In the rats, after exposure to the high concentration, bronchial fibrosis occurred in the lungs in the form of intraluminal and intramural, polypoid protrusions (Morgan et al. 2012 b).

The fibrotic lesions in the rats were examined histopathologically and described as follows in Morgan et al. (2012 b): Fibrotic lesions originated from the lamina propria of the bronchi, evidently at the sites with epithelial necrosis and ulceration. The intraluminal polyps were often found to have at least partial ulceration of the overlying epithelium, epithelial inclusions within the bronchial wall and lymphatic infiltrates at the base. The fibroblasts within these polyps were aligned vertically to the bronchial base of the lesion, suggesting movement towards the damaged epithelium. Some of the fibrotic lesions produced only sessile, mound-like elevations of the ulcerated bronchial surfaces, protruding into the lumen. In addition to polypoid fibrosis, foci of epithelial necrosis and ulceration without associated fibrosis were found in the large bronchi and some of the bronchial branches had fibrosis extending round their circumference with an intramural or constrictive structure, resulting in thickening of the bronchial walls and probably a reduction in the diameter of the lumen. This intramural type of fibrosis was noted more often in the bronchial branches than in the major airways. Fibroplasia and inflammatory infiltrates were likewise present in the adventitial layer of many of the bronchi with intramural fibrosis. These lesions of the lungs correspond to the characteristics of bronchiolitis obliterans in humans.

In a comparative study, groups of 3 to 9 male Wistar rats were exposed to 2,3-pentanedione concentrations of 0, 100, 150 or 200 ml/m³ for 6 hours a day, on 5 days a week, for 12 days. The recovery periods were one day or two weeks. Under the same conditions, rats were exposed to diacetyl and the histopathological results were compared. Exposure to diacetyl led to weaker effects than exposure to 2,3-pentanedione, with intraluminal and intramural bronchial fibrosis in 3 of 5 animals at 200 ml diacetyl/m³ and in 1 of 6 animals at 150 ml diacetyl/m³ (2,3-pentanedione: 5/5 and 3/6, respectively, see Table 1). After the 2-week recovery period, no differences in the number of bronchial lesions after exposure to diacetyl or 2,3-pentanedione were observed. Also the lesions in the nose and the larynx caused by the two substances were similar (no other details; Morgan et al. 2012 a). The results are given only in an abstract; a more detailed publication of the studies was not found.

In a study investigating gene expression in lesions similar to bronchiolitis obliterans, 5 male Wistar-Han rats were whole-body exposed to 2,3-pentanedione at a concentration of 200 ml/m³ for 6 hours a day, on 5 days a week for 12 days. All exposed animals developed bronchial lesions with fibrosis, epithelial ulceration, regeneration, hyperplasia and inflammation. Analysis of the gene expression in bronchial fibrotic tissue of the exposed animals revealed the participation of more than 3800 genes, including those coding for a large number of known profibrotic mediators, and also the up-regulation of genes involved in vascular development and remodelling of the extracellular matrix (ECM). These include proteases and their inhibitors, which participate in the regulation of ECM. In addition, the cytokine Tgf-β2 (transforming growth factor β2), a key cytokine in tissue repair and fibrosis, which has already been associated with bronchiolitis obliterans in lung transplant patients, and the interleukins (IL-1α, IL-24, IL-33, IL-18), as further profibrotic mediators, were up-regulated (Morgan et al. 2015).

The lesions in the lungs are described in Morgan et al. (2015) as follows: The bronchial fibrotic lesions were mainly of the intraluminal plaque-like or polypoid type, and occasional lesions were confined to the bronchial wall (intramural). The stroma of the fibrosis was loose and myxoid and contained mucopolysaccharides but little mature collagen. Bronchial and peribronchial inflammatory infiltrates, composed of histiocytes, lymphocytes, eosinophils and occasionally neutrophils were present in the areas of the bronchial fibrosis as well as in some non-fibrotic areas. Bronchial epithelial ulceration, with fibrin-deposits on the surface was often seen in the larger bronchi, and was sometimes extensive. A variety of bronchial epithelial changes were noted in non-ulcerated airways, including reactive and regenerative epithelial changes and epithelial hyperplasia; epithelial hypertrophy and hyperplasia were also seen in some of the bronchioles. Bronchial-associated lymphatic tissue (BALT) was hyperplastic in some animals, particularly in areas of fibrosis. The lungs of control animals were inconspicuous, except for occasional foci of perivascular and peribronchial eosinophils and mononuclear cells. These airway lesions are histopathologically similar to the airway lesions found with bronchiolitis obliterans in humans.

A comparison of inhalation studies in rats and mice (Table 2) reveals that the effects of the α-diketones 2,3-pentanedione and diacetyl in the nasal cavity and, at higher concentrations, also in the lungs are very similar in both rodent species. For diacetyl, benchmark concentrations calculated by the NIOSH (2013) were also included. The benchmark concentrations for the rats were calculated from the data given in a study of the National Toxicology Program, which are not accessible. The lowest observed adverse effect concentrations (LOAECs) were not given.

Tab. 2 Comparison of the effects of the α-diketones 2,3-pentanedione and diacetyl after inhalation exposure of rats and mice

Species	2,3-Pentanedione		References	Diacetyl		References
Effects in the nose						
rat	2 weeks	**49 ml/m³: LOAEC** nasal cavity: inflammation, exudate, metaplasia (minimal to mild), olfactory epithelium: atrophy, degeneration (minimal)	Morgan et al. 2012 b	13 weeks (0, 6.25, 12.5, 25, 60, 100 ml/m³) no other details	**LOAEC not given**, effects on larynx, nose and trachea, olfactory epithelium: degeneration, **BMC: 20 ml/m³**	NIOSH 2013
mouse	2 weeks	**49 ml/m³: LOAEC** nasal cavity: inflammation, exudate, respiratory epithelium: necrosis, metaplasia, regeneration (minimal to mild), olfactory epithelium: atrophy (mild), larynx: inflammation, necrosis	Morgan et al. 2012 b	6 weeks	**25 ml/m³: LOAEC** nasal cavity: inflammation, exudate, respiratory epithelium: metaplasia (minimal to mild)	Morgan et al. 2008
				12 weeks	**25 ml/m³: LOAEC** nasal cavity: inflammation (minimal to mild), respiratory epithelium: metaplasia (minimal), respiratory epithelium: necrosis, **BMC: 17 ml/m³** (NIOSH 2013)	Morgan et al. 2008

Tab. 2 *(continued)*

Species	2,3-Pentanedione		References	Diacetyl		References
Effects in the lungs						
rat	2 weeks	**150 ml/m³:** bronchial fibrosis	Morgan et al. 2012 a	2 weeks	**200 ml/m³:** bronchial fibrosis	Morgan et al. 2012 a
	2 weeks	**97 ml/m³:** regeneration of the bronchial epithelium (12/12, minimal)	Morgan et al. 2012 b	13 weeks, (0, 6.25, 12.5, 25, 60, 100 ml/m³) no other details	**LOAEC not given**, eosinophilic inflammation, **BMC: 29 ml/m³**	NIOSH 2013
mouse	2 weeks	**49 ml/m³: LOAEC** inflammation (minimal)	Morgan et al. 2012 b	6 weeks	**25 ml/m³: LOAEC** peribronchial inflammation (minimal)	Morgan et al. 2008
				12 weeks	**25 ml/m³: LOAEC** peribronchial inflammation (minimal), bronchus: chronic inflammation, **BMC: 19 ml/m³** (NIOSH 2013)	Morgan et al. 2008

BMC: benchmark concentration for 10% increase over controls; LOAEC: lowest observed adverse effect concentration

In the case of the β-diketone 2,4-pentanedione on the other hand, the main effects are neurotoxic effects; here, no effects in the nasal cavity or lungs were described. The increased expression of IL-6 and NO-synthase-2 and the decreased expression of the growth factor Vegf-A in various brain regions after exposure to 2,3-pentanedione indicate that this substance can, in very high concentration ranges, also have neurotoxic effects (Greim 2007, available in German only; Hubbs et al. 2012).

Table 3 shows a comparison of the histopathological findings after exposure to 2,3-pentanedione and diacetyl with the histopathology of bronchiolitis obliterans occurring in workers in the popcorn industry (see Section 4.2). The effects in the lung tissue of rats after exposure to 2,3-pentanedione are similar to those in patients with bronchiolitis obliterans, whereas no such lesions occur in exposed mice.

Tab. 3 Comparison of histopathological findings in the lungs

2,3-Pentanedione	Diacetyl	Exposure in popcorn industry
110 ml/m³, 6 hours, rats: pycnosis and caryorrhexis of some respiratory epithelial cells in the stem bronchus (Hubbs et al. 2012)		**humans (bronchiolitis obliterans)**: time course: inflammation – myofibroblast activity – fibrin deposition
150 ml/m³, 2 weeks, rats: intramural and intraluminal fibrosis (Morgan et al. 2012 a; NIOSH 2013)		submucosal fibrosis after inflammation, narrowing of the bronchiolar lumen, intraluminal formation of granulation tissue (European Commission 2014)
200 ml/m³, 2 weeks, rats: bronchial fibrosis in the form of intraluminal, polypoid protrusions, fibrosis with intramural or constrictive patterns, foci with necrosis and ulceration in the large bronchi without fibrosis, thickened bronchial wall (Morgan et al. 2012 b)	**200 ml/m³, 2 weeks, rats**: intramural and intraluminal fibrosis (Morgan et al. 2012 a; NIOSH 2013)	

Tab. 3 *(continued)*

2,3-Pentanedione	Diacetyl	Exposure in popcorn industry
200 ml/m³, 2 weeks, rats: intraluminal or polypoid fibrosis of the bronchi; intramural lesions, bronchial epithelial protrusions with sometimes extensive areas containing fibrin on the surface in the large bronchi (Morgan et al. 2015)		
200 ml/m³, 2 weeks, mice: fibrotic lesions of the bronchi with necrosis and ulceration (Morgan et al. 2012 b)	**200 ml/m³, 12 weeks, mice**: no necrosis of the mucosa in the extrapulmonary or intrapulmonary bronchi (Morgan et al. 2008); **400 ml/m³, 12 weeks, mice**: extensive coagulative necrosis of the mucosa in the trachea and bronchi (extrapulmonary), necrosis of large intrapulmonary bronchi, degenerative changes in the epithelium of the stem bronchus, no lesions of the bronchioles or pulmonary parenchyma (Morgan et al. 2008)	

Summary: After rats were exposed to 2,3-pentanedione for 12 days, inflammation, exudate and metaplasia in the nasal cavity and minimal effects on the olfactory epithelium occurred even at the lowest concentration tested of 49 ml/m³ and increased concentration-dependently. Initial pulmonary effects were observed at 97 ml/m³ and above, with a no observed adverse effect concentration (NOAEC) of 49 ml/m³ for the lungs. Decreased body weights and effects in the larynx were found in addition at the highest concentration tested of 202 ml/m³.

In mice exposed for 12 days, effects were found in the nasal cavity, olfactory epithelium, larynx and lungs even at the lowest concentration tested of 49 ml/m³.

A NOAEC of 49 ml/m³ was thus obtained for effects on the lungs in rats, and a LOAEC of 49 ml/m³ for effects on the nose in rats, and for effects on the lungs and nose in mice.

Histopathological examination of the lung tissue of rats after 12-day exposure to 2,3-pentanedione revealed intramural and intraluminal fibrosis, which are also characteristic of the disease bronchiolitis obliterans in humans.

5.2.2 Oral administration

There are no data available.

5.2.3 Dermal application

There are no data available.

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

The 24-hour application of undiluted 2,3-pentanedione to the intact or shaved rabbit skin under occlusive conditions led to moderate irritation (no other details; Author not named 1979 b).

5.3.2 Eyes and mucous membranes

There are no studies available for the effects of 2,3-pentanedione on the eyes.

Exposure of male Sprague Dawley rats to 2,3-pentanedione concentrations of 320 or 360 ml/m³ for 6 hours increased the reactivity of the mucous membranes of isolated, perfused trachea preparations treated with methacholine (Zaccone et al. 2013).

5.4 Allergenic effects

5.4.1 Sensitizing effects on the skin

A publication describes studies using the local lymph node assay in BALB/c mice with 2,3-pentanedione and three other α,β-dicarbonyl compounds. A 4:1 mixture of acetone and olive oil was used as the vehicle. A concentration of 15.4% was determined for 2,3-pentanedione which produced a 3-fold increase in lymphocyte proliferation (EC3 value). For 2,3-hexanedione, 3,4-hexanedione and 2,3-heptanedione, the values were similar at 18.2%, 15.5% and 14.1%, respectively. To evaluate the irritant effects, the increase in ear thickness was determined in a separate experiment with the four substances. In concentrations up to 50%, none of the substances were found to be irritating. After topical application, in the mouse model the percentage of B220+ cells in the lymph nodes increased markedly compared with the vehicle controls. According to the authors this indicates a contact sensitizing potential of 2,3-pentanedione (Anderson et al. 2013). In this study, diacetyl, already described in an earlier publication as a contact allergen, was also investigated. In contrast to the earlier study (Anderson et al. 2007; Hartwig and MAK Commission 2016), however, markedly higher EC3 values (14.2%, 17.9% and > 25%) were determined for three new diacetyl samples than with the original sample (EC3: 2.5%). In this sample, the authors demonstrated the presence of a dimeric impurity, which in the three other samples was present only in a much lower concentration. This impurity is the product of aldol condensation between two diacetyl molecules. However, the authors did not investigate the sensitizing properties of this impurity (Anderson et al. 2013). The primary reaction product can, however, under renewed, now intramolecular aldol condensation and water cleavage finally react to form 2,5-dimethylbenzoquinone. The corresponding 2,5-diethylbenzoquinone is also contained in 2,3-pentanedione as an impurity. As EC3 values of approximately 0.01% were determined for the unsubstituted benzoquinone (Roberts and Aptula 2009), the substituted benzoquinones formed from diacetyl and 2,3-pentanedione could be responsible for the observed contact sensitization.

In an NTP study only available in the form of an abstract, 2,3-pentanedione concentrations between 5% and 25% produced at least a 3-fold increase in lymphocyte proliferation in the local lymph node assay. Although a significant increase in lymphocyte stimulation was obtained using a 2.5% preparation, the increase was below 3 times the value of the vehicle control response. In this study, 2,3-pentanedione was not irritating up to concentrations of 25% (ECHA 2020). This result indicates a slight to moderate contact allergenic potential of 2,3-pentanedione. This study was cited as “NTP 2011” in the German documentation, however, it is no longer available from the website of the NTP.

The following tests were described in the German documentation and also cited as “NTP 2011” but are no longer available from the website of the NTP. The findings obtained with 2,3-pentanedione in a mouse ear swelling test, which is likewise available only in the form of an incompletely documented abstract, can also not be evaluated unequivocally. In mice pretreated with 10% or 25% 2,3-pentanedione, challenge with a 25% preparation produced significantly more pronounced swelling of the ear after 24 and 48 hours than in the control group pretreated only with the vehicle. In this group, when compared with the group treated only with the vehicle in the challenge, a significant increase in ear thickness was, however, likewise observed. In another study, pretreatment was carried out with 5% to 25% 2,3-pentanedione. The increase in ear thickness determined after challenge with the 25% preparation was, however, not statistically significant compared with the ear thickness in the control group.

5.4.2 Sensitizing effects on the airways

Only one in vivo investigation of respiratory sensitization is available; topical application of 25% and 50% 2,3-pentanedione in mice did not produce a concentration-dependent increase in total IgE (Anderson et al. 2013).

5.5 Reproductive and developmental toxicity

There are no data available.

5.6 Genotoxicity

5.6.1 In vitro

In bacterial mutagenicity tests with the Salmonella typhimurium strains TA98, TA100 and TA102, 2,3-pentanedione was not found to be mutagenic in the concentration range of 0.009 to 900 µmol/plate in the presence and absence of a metabolic activation system (S9 rat liver mix induced with Aroclor 1254) (Aeschbacher et al. 1989; Kim et al. 1987). The validity of the study of Kim et al. (1987) cannot be evaluated due to the lack of details.

The presence of 105 µmol 2,3-pentanedione/plate suppressed the mutagenicity of mutagenic heterocyclic amines (TA100) (Kim et al. 1987).

EFSA (2014) listed 13 studies for diacetyl, some of which yielded a positive result. However, only one of these studies was considered by the EFSA as valid. In the study of Aeschbacher et al. (1989), diacetyl was found to have only limited mutagenicity (Aeschbacher et al. 1989; EFSA 2014).

5.6.2 In vivo

In a study with groups of 5 male and 5 female Wistar Han rats and B6C3F1 mice, no micronuclei were found in polychromatic and normochromatic erythrocytes in the peripheral blood 24 hours after the end of 13-week inhalation exposures to 2,3-pentanedione concentrations of 0, 6.25, 12.5, 25, 50 or 100 ml/m^3 (5 days/week). No cytotoxicity was observed (NTP 2010).

5.7 Carcinogenicity

There are no data available.

5.8 Other effects

2,3-Pentanedione inhibited the cell growth of BP8 cells (“ascites sarcoma”) in vitro by 100% at a concentration of 1 mM, while a concentration of 0.1 mM led to inhibition by 34% (Pilotti et al. 1975).

TLR-4 (toll-like receptor-4) is up-regulated in patients with lung fibrosis as a secondary reaction after chronic inflammation. In vitro, 2,3-pentanedione and other diketones were not agonists of this receptor and also did not display synergistic activity (Kerger et al. 2014).

6 Manifesto (MAK value/classification)

The critical effects of 2,3-pentanedione are irritation and inflammatory processes in the nasal cavity and the lungs after 12-day exposure of rats and mice, which are similar to those obtained with diacetyl (Hartwig and MAK Commission 2016).

After the exposure of workers to the structural analogue diacetyl, subclinical changes in lung function, irreversible airway obstruction and bronchiolitis obliterans were observed as the critical effects.

MAK value. There are no data available for the effects of 2,3-pentanedione in humans. Therefore, for the derivation of a MAK value, animal studies are used and the structural similarity to diacetyl is taken into account.

In a 12-day inhalation study in rats and mice with 2,3-pentanedione, inflammation and exudate in the nasal cavity, metaplasia in the respiratory epithelium and minimal effects in the olfactory epithelium were found in rats even at the lowest concentration tested of 49 ml/m³. These effects increased in a concentration-dependent fashion. The mice were more sensitive and in addition to the effects in the nasal cavity and the olfactory epithelium also inflammation and necrosis in the larynx were observed at the lowest concentration tested of 49 ml/m³; in 9 of 12 animals, there was also minimal inflammation of the lungs. Both the incidence and severity of these effects were concentration-dependent (Morgan et al. 2012 b).

The occurrence of fibrosis in rats after only 12 exposures to 2,3-pentanedione is considered to be of particular significance.

The nasal breathing of rodents is the reason for the preferential occurrence of effects in the nasal cavity of rodents, whereas breathing through the mouth during physical exertion could be a reason for the more intense effects in the lungs in humans.

In rats, a NOAEC of 49 ml/m³ for lung effects was obtained. Without the availability of a 90-day study it cannot be excluded that the no adverse effect concentration (NAEC) after long-term exposure is very much lower (1:6). From a comparative flow simulation model with diacetyl, the exposure of the bronchioles in humans, with a respiratory volume of 25 l/min, was calculated to be up to 40 times higher than that for rats (Gloede et al. 2011; Hartwig and MAK Commission 2016). Because of its structural similarity to diacetyl, this is also assumed for 2,3-pentanedione. For the derivation of a MAK value, a respiratory volume of 20.8 l/min is assumed; humans working at the workplace would therefore be exposed at a level that is a maximum 32 times higher than that in the rat. From the NOAEC in the rat, therefore, a NAEC of about 0.2 ml/m³ is obtained for humans for effects in the lungs.

The studies with mice yielded a LOAEC of 49 ml/m³ for effects in the lungs. As the effects were only slight, a NAEC of 16 ml/m³ (LOAEC/3) is assumed. Without the availability of a 90-day study, it cannot be excluded that the NAEC after long-term exposure is lower (1:6). In addition, the higher exposure of the bronchioles in humans from breathing through the mouth and an increased respiratory volume compared with that in mice (NIOSH 2013) (1:12) must be taken into account. Therefore, a NAEC of about 0.2 ml/m³ for humans is derived from the data with mice.

In a 12-week inhalation study with mice, the structural analogue diacetyl likewise induced effects in the respiratory and olfactory epithelium and inflammatory processes in the pulmonary epithelium at concentrations similar to the 2,3-pentanedione concentrations used. Calculations carried out by the NIOSH (2013) of the relative toxic potency of 2,3-pentanedione and diacetyl show that their toxicity is similar.

Bronchiolitis obliterans, an irreversible lung disease occurring in workers after exposure to diacetyl, was observed after exposure to markedly lower mean concentrations. Therefore, diacetyl is assumed to have a cumulative effect (Hartwig and MAK Commission 2016).

The results of the histopathological examinations of the lungs in the animal studies have been interpreted to mean that 2,3-pentanedione and diacetyl have similar phenomenological features. The intraluminal and intramural fibrosis observed in rats correspond to the histopathological observations obtained in the lungs of humans in cases of bronchiolitis obliterans. The critical effects found after the exposure of workers to diacetyl were subclinical changes in lung function, airway obstruction and bronchiolitis obliterans. The structural similarity of 2,3-pentanedione and diacetyl allows the conclusion that 2,3-pentanedione leads to similar toxic effects in humans.

The MAK value for diacetyl of 0.02 ml/m³ was derived from the no observed adverse effect levels (NOAELs) for the reduced one second capacity in exposed workers of 0.8 and 0.65 ml/m³ × years ("ppm years") and extrapolated to 40 years. In analogy, a MAK value of 0.02 ml/m³ has likewise been established for 2,3-pentanedione.

Peak limitation. Although the effects occur at the port of entry of the lungs, but are not an immediate irritant effect, the substance is classified in Peak Limitation Category II. In analogy to diacetyl, an excursion factor of 1 has been established.

Prenatal toxicity. There are no studies available for the developmental toxicity of 2,3-pentanedione. The substance is therefore classified in Pregnancy Risk Group D.

As no developmental toxicity was observed up to the highest dose tested of 1600 mg/kg body weight and day with the structurally similar diketone diacetyl (2,3-butanedione) in rats, mice and hamsters, the classification of 2,3-pentanedione therefore tends towards Pregnancy Risk Group C.

Carcinogenicity and germ cell mutagenicity. There are no studies available for the carcinogenicity of the substance. In the Salmonella strains TA98, TA100 and TA102, 2,3-pentanedione was not found to be mutagenic with or without the addition of a metabolic activation system. The results of micronucleus tests in the erythrocytes of rats and mice were negative. The substance is not classified in one of the categories for carcinogens. As, all in all, there is no evidence of germ cell mutagenicity, the substance is not classified in one of the categories for germ cell mutagens.

Absorption through the skin. Neither in vitro nor in vivo data are available for the absorption of 2,3-pentanedione through the skin. Using model calculations, dermal absorption from a saturated aqueous solution of the compound was estimated to be a maximum 96.2 mg under standard conditions.

With an LD_{50} > 2500 mg/kg body weight, the acute dermal toxicity is low. In an inhalation study with rats and mice, a LOAEC of 49 ml/m³ (203 mg/m³) was obtained after 12 exposures for the decrease in body weights. With an estimated NAEC (1:3) of 16 ml/m³ (68 mg/m³) and assuming a possible increase in the effects over time (1:6) as well as an increased respiratory volume of workers at the workplace (1:2), and taking into consideration extrapolation from animals to humans, a systemically tolerable amount of 28 mg can be derived.

As the estimated maximum absorbable quantity through the skin is above this value, dermal absorption is assumed to make a relevant contribution to the systemic toxicity of the compound. 2,3-Pentanedione is therefore designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts). The structural analogue diacetyl has likewise been designated with an "H".

Sensitization. There are no clinical findings available for the contact sensitizing properties of 2,3-pentanedione. The positive results from two independent studies using the local lymph node assay in mice and the mouse ear swelling test indicate a low potential for contact sensitization, as is also the case with diacetyl which has skin sensitizing effects in humans. It is not clear whether this contact sensitization is based on the formation of reactive condensation products. As regards the respiratory sensitization of 2,3-pentanedione, no findings in humans, and no useful results from experimental animal studies are available. Based on the positive results from experiments on the skin of mice and taking into consideration the clinical findings obtained with diacetyl, which is closely related in structure, 2,3-pentanedione is therefore designated by analogy with "Sh" (for substances which cause sensitization of the skin), but not with "Sa" (for substances which cause sensitization of the airways).

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